- Primers should contain at least one CpG site within their sequence, and the CpG site should preferably be located in the most 3'-end of their sequence to discriminate methylated DNA against unmethylated DNA.
- Primers should have a minimal number of non-CpG cytosines in their sequence to amplify only bisulfite converted DNA. Primers with more non-CpG cytosines are preferred, since the bisulfite conversion may on some occasions be incomplete.
- The set of primers for methylated DNA and the set for unmethylated DNA should contain the same CpG sites within their sequence. For example, if a forward primer for methylated DNA has this sequence: ATTAGTTTCGTTTAAGGTTCGA (SEQ ID NO:15), the forward primer for unmethylated DNA must also contain the two CpG sites as the methylated forward primer. However, they may differ in length and start position.
- Both sets of primers should have similar annealing temperature.

Page 17, replace the paragraph between lines 1 – 8 with the following:

The corresponding reference assay was performed using the following primers ~~and probes~~:Primer: TCCATATTCCAAACCCTATACCAAA (SEQ ID NO:13); Primer: TGGGATTGAGGGTAAGAGGGAT (SEQ ID NO:14). The reaction is run with the following assay conditions: *Reaction solution*: (900 nM primers; 300 nM probe; 3.5 mM Magnesium Chloride; 1 unit of taq polymerase; 200 µM dNTPs; 7µl of DNA, in a final reaction volume of 20µl);*Cycling conditions*: (95°C for 10 minutes; then 50 cycles of: 95°C for 15 seconds; 60°C for 1 minute ). The reaction is observed in real time by use of commercially available instruments such as the ABI PRISM 7700 sequence detector.